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The Effect of Pure Infrared Light on the Growth of Rhodospirrilum rubrum

Jordan Lee Wilkes
Utah State University

THE EFFECT OF PURE INFRARED LIGHT ON THE GROWTH OF *RHODOSPIRRILUM RUBRUM*

by

Jordan Lee Wilkes

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of the requirements for the degree

of

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Approved:

Thesis/Project Advisor	**Departmental Honors Advisor**
Dr. Scott A. Ensign	Dr. Alvan Hengge

Director of Honors Program
Dr. Kristine Miller

UTAH STATE UNIVERSITY
Logan, UT

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Abstract

Scientists who study aquatic ecosystems quickly notice a diversity of pathways that different microbes and organisms can use to metabolize nutrients found in common ponds or pools. Competition for vital resources, such as light and inorganic minerals, allow only certain organisms to grow in certain niches within these ecosystems. *Rhodospirillum rubrum* is a gram negative, photosynthetic bacteria that competes for light within aquatic ecosystems in order to survive. R. rubrum is believed to specifically absorb light for photosynthesis at wavelengths in the range of infrared light. It was found that *R. rubrum* indeed can grow in "dark", anaerobic environments by being grown purely on infrared light. This was facilitated in the lab by growing *R. rubrum* on pure infrared light and comparing the absorbance of these cultures with a control of *R. rubrum* grown using an incandescent light bulb (visible light). Because of these findings, it is believed that *R. rubrum* can grow in ponds and other aquatic environments where organic filters, such as algae pollution, have absorbed all visible light for photosynthesis. Furthermore, our data supports the fact that *R. rubrum* could hypothetically grow entirely off of infrared light in aquatic environments. Strains of unknown bacteria have been found to grow on pure infrared light with acetone as the carbon source.

Acknowledgements

The accomplishments of an undergraduate student are not only the product of the hard work of the student, but also of countless other people who have taken the time to work with, mentor, teach, guide, and support the student along the way.

I want to first thank Dr. Scott Ensign of the Department of Chemistry and Biochemistry. There is no greater mentor at Utah State University. Dr. Ensign was the brains behind every project and experiment conducted in the lab. It was his inspiration when I was in high school that helped me decide to pursue a degree in Biochemistry. His time taking us into the lab made all of the difference in the world in regards to my undergraduate studies.

Next, I want to thank the countless others who worked with me and supported me in the lab. These individuals include Tanner Godfrey, Emily Avila, and most especially Namish Khadka. Namish has become a great friend and colleague as he has helped me in the lab and mentored me when I had questions or needed to receive practical training.

Support has also come from professors and advisors too numerous to mention here. I want to extend a special thank you to these individuals for their support and inspiration in opening my eyes to the world of science. Many of these individuals manifested their help through organizations. Thank you to the Honors Department for guiding me along the path to my thesis and making this all possible. Funding for this research was provided by the College of Science through a Mini-Grant that was awarded. Thank you to the College for your support of undergraduate research.

And last but certainly not the least, I want to thank my beautiful wife Kelsie for patiently sitting around waiting for me to finish my homework or submit that last assignment. Your love and support is what keeps me going to achieve my dreams and to better my own life. I love you and thank you for your support!

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Abbreviations

- NADPH (Nicotinamide adenine dinucleotide phosphate)
- ATP (Adenosinde triphosphate)
- A. caulinodans (Azorhizobium caulinodans)
- R. rubrum (Rhodospirillum rubrum)
- nm (nanometer)
- mL (milliliter)
- g (gram)
- mg (milligram)
- O_2 (atmospheric oxygen)
- P680/P700/P870 (Denotes a photosystem and the absorbable wavelength of light in nanometers)
- C (Celsius)
- EDTA (Ethylenediaminetetraacetic acid)
- NH_4Cl (Ammonium chloride)
- $CaCl_2$ (Calcium chloride)
- Na_2MoO_4 (Sodium molybdate)
- P_i (Inorganic phosphate)
- $CoCl_2x6H_2O$ (Cobalt chloride hexahydrate)
- $MnCl_2xH_2O$ (Manganese chloride monohydrate)
- $CuCl_2x2H_2O$ (Copper chloride dehydrate)
- $ZnSO_4x7H_2O$ (Zinc sulfate heptahydrate)
- $NiCl_2xH_2O$ (Nickel chloride monohydrate)
- $VOPO_4xH_2O$ (Vanadyl phosphate monohydrate)
- $Na_2WO_4xH_2O$ (Sodium tungstate monohydrate)
- Na_2SeO_3 (Sodium selenite)
- Na_2EDTA (Sodium ethylenediaminetetraacetic acid)
- ddH_2O (distilled water)
- $MgSO_4x7H_20$ (Magnesium sulfate heptahydrate)
- H_3BO_3 (Boric Acid)
- Fe (III) citrate (Iron citrate)
- MOPS (3-[N-morpholino]propanesulfonic acid)
- KH_2PO_4 (Potassium dihydrogen phosphate)
- Ar (Argon gas)
- CO_2 (Carbon dioxide)
- DTT (Dithiothreitol)
- IDS (Indigo carmine)
- mM (Millimolar)
- $NiCl_2$ (Nickel chloride)
- A_{600} (Absorbance at a wavelength of 600 nanometers)
- CO (Carbon monoxide)

Introduction

Research in the Ensign Lab focuses on the biochemical processes that support metabolism and life. Microbes utilize materials commonly found in the environment to survive. Using enzymatic reactions, bacteria are able to generate metabolites such as NADPH and ATP which are vital for survival.

My work started with an exploration into the metabolic processes of *Xanthobacter* bacteria. *Xanthobacter* utilizes gases such as propylene to produce metabolites in the cell.[1] This is done by using a molecule called Coenzyme M. *Xanthobacter* was grown as a control. Bacterial cultures grown using inoculants from the environment (these will be referred to as enrichments) were created using rotting fruit as a source of the carbon gases necessary for growth. The enrichments were created in hopes of isolating new strains of bacteria that use similar metabolic processes as *Xanthobacter*.

Next, we focused our studies on the enzyme nitrogenase. This enzyme, which we tested in *Azorhizobium caulinodans (A. caulinodans)*, fixes atmospheric dinitrogen into ammonium which is incorporated into metabolites that are consumed by other organisms.[2] *A. caulinodans* was isolated from nodules on the roots of plants that require nitrogen such as legumes. A de-repression assay was used to measure the activity of nitrogenase. This assay is based on the fact that nitrogenase is promiscuous and will reduce the triple bonds of molecules other than dinitrogen. Acetylene was added to growths of the bacteria and monitored over time for the production of ethylene using a gas chromatograph. These preliminary studies familiarized me with how to handle bacteria and how to monitor the growth and/or activity of the cultures. These experiments were in preparation for work on photosynthetic bacteria.

The many metabolic mechanisms of microbes and other organisms particularly in aquatic ecosystems, use a diverse set of pathways to metabolize nutrients. Competition for vital resources, such as light and inorganic minerals, allow only certain organisms to grow in certain niches within these ecosystems. *Rhodospirillum rubrum (R. rubrum)* is a gram negative, purple-non-sulfur-photosynthetic bacteria that competes for light within aquatic ecosystems in order to survive.[3, 4] *R. rubrum* is believed to specifically absorb light for photosynthesis at wavelengths in the range of infrared light (840-870 nm).[5, 6] It was found that *R. rubrum* indeed can grow in "dark", anaerobic environments purely on infrared light. This was explored by comparing the growth of *R. rubrum* on pure infrared light versus an incandescent light bulb (visible light) which has been the common method of growing *R. rubrum* in the lab. To understand these processes, one must be familiar with some specifics about photosynthesis.

Photosynthesis is the process of converting light energy into chemical energy for a cell. This has been well studied in plants and algae which are thought of as the main photosynthesizers in the global ecosystem.[7] Plants and algae are able to perform these functions through organelles called chloroplasts which are early endosymbionts with plant cells (much like mitochondria).[8] These are located within the cytosol of the cell. Chloroplasts contain photosystems which are an assortment of light absorbing biomolecules called chlorophyll (which gives plants their distinctive green color). These chlorophyll absorb energy which is passed off to the reaction center of the photosystem. The reaction centers absorb the light energy which causes a charge separation in the reaction center. This in turn excites an electron which is passed through several molecules in a process called the Z scheme (see Figure 1). The electrons in these photosystems are replenished by splitting water. The electrons are eventually passed to a second photosystem. This is accomplished by an assortment of enzymes called the O_2 Evolving Complex. This photosystem

absorbs light at a slightly different wavelength which excites an electron. This electron is again passed along various biomolecules until it ends up on a reduced NADPH. This NADPH molecule is known as a Terminal Electron Acceptor (TEA). This movement of electrons is accompanied by a pumping of protons across the membrane of the chloroplast. This creates a proton gradient which is used to fuel the production of ATP (high energy molecules used by the cell for metabolism) by ATP synthase.

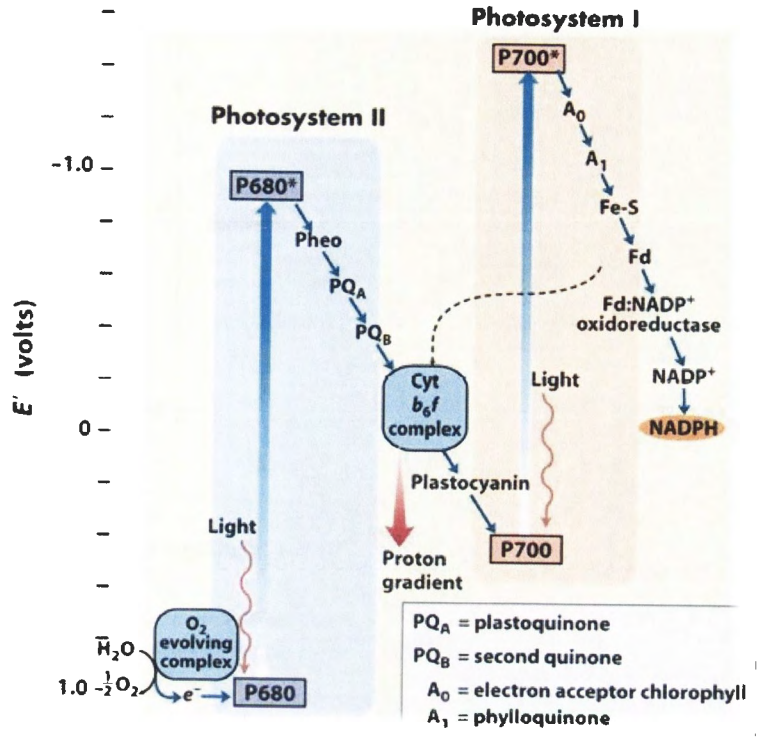


Figure 1. A rendition of the Z scheme which plants and algae use as part of photosynthesis. This is also called non-cyclic photosynthesis.[7]

Some bacteria are also able to photosynthesize by using reaction centers located within the plasma membranes of the cells. The plasma membranes of this class of bacteria create folds which bend into sheets to increase the surface area of the plasma membrane.[9] Though the general idea of turning light energy into chemical energy is the same, there are very large differences between photosynthesis in plants and in bacteria. First, bacteria use bacteriochlorophyll in contrast to chlorophyll. This difference helps gives bacteria a characteristic color when they are grown on light. These bacteria also do not use water as the source of electrons to fuel photosynthesis. Instead sulfur or another carbon-oxygen source (such as malate or carbon monoxide) is used to absorb the light and become excited. One final difference between bacterial and plant photosynthesis is that bacteria use only one photosystem instead of two linked photosystems. The electron that is excited is ultimately passed along back to the initial photosystem. This is known as cyclic photosynthesis and can be used by plants when a high level of ATP is needed in the cell. Because of this, bacteria use photosynthesis strictly to produce ATP when this is needed. Another major result of cyclic photosynthesis is that oxygen is not produced by the splitting of water. These photosynthetic bacteria only use photosynthesis for metabolism when they are growing anaerobically.

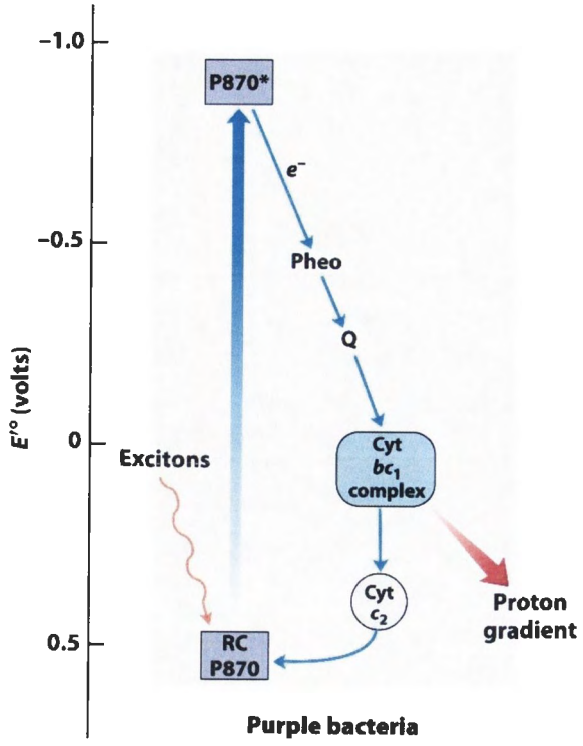


Figure 2. A rendition of cyclic photosynthesis which is carried out in *R. rubrum* as well as many other bacteria.[7]

There are very specific requirements needed for bacteria to use photosynthesis to grow. *R. rubrum* is a purple-non-sulfur-photosynthetic bacteria that has been isolated from water systems such as lakes and ponds. *R. rubrum* is a very distinctive purple-red color when anaerobic (due to the bacteriochlorophylls) and is a facultative anaerobe meaning that it can survive in environments with or without oxygen. When in an aerobic environment, *R.* rubrum is colorless and uses fermentation to make ATP for survival. These qualities make it very easy to identify and also very easy to isolate from enrichments such as pond water or mud.

Despite its range of abilities and metabolic techniques (ranging from the use of nitrogenase to fermentation and the production of sulfur), we focused specifically on *R. rubrum's* photosynthetic abilities.[10] *R. rubrum* has the ability to absorb light at varying wavelengths due to the specific bacteriochlorophylls that it contains. *R. rubrum* can grow on light in the longer ranges of the visible spectrum and into the infrared spectrum as well. This gives *R. rubrum* a spectrum of photosynthetic absorption from roughly about 650 nm to upwards of 950 nm.[5]

Understanding this helps us to hypothesize how *R. rubrum* is able to grow anaerobically. Here we show that by using our knowledge of the photosynthetic capabilities of *R. rubrum*, we can utilize light emitted at specific wavelengths to determine how this affects growth of the bacteria. The usefulness of this data can be understood by remembering that *R. rubrum* was isolated from a mud culture. In many pond ecosystems, plants and algae could layer the top of a pond and thrive by using the visible light energy and the oxygen readily available to them on the surface. The algae would create a biological filter that could only let light other than visible light pass through to organisms below the surface. In these anaerobic conditions, *R. rubrum* could thrive by absorbing and utilizing infrared light that passes through the algae. Our research shows that *R. rubrum* grows on pure infrared light just as well as on visible light.

Methods and Materials

In the Ensign Lab, *R. rubrum* has been grown using an incandescent light bulb that emits various wavelengths of light including visible and infrared. This was changed to test the growth of *R. rubrum* under varying conditions. The bacteria was taken from a freezer stock of cells which was 20% glycerol by volume kept at -80°C. The cells were allowed to thaw before being inoculated for growth. Proper sterile technique was employed by using a Laminar flow hood. All surfaces were wiped down with ethanol before and after each use and open Pyrex containers were flamed with a Bunsen burner around the rim to ensure no contamination of any of the samples. Sterile pipettes were used for all transferring mentioned in these methods.

The thawed cells were placed in a liquid media that contained 1000X Trace Elements, biotin, EDTA, NH_4Cl, $CaCl_2$, and Na_2MoO_4. The exact amounts of these ingredients were unknown as they were stock solutions previously created in the lab following the lab's general protocol. It is uncertain if the protocol was exactly followed. The pH of the media was adjusted to 7 and then the media was autoclaved along with a 40X phosphate source (P_i). The inoculations were performed in 23 mL test tubes that had screw-on caps on them to ensure anaerobic growth. To these tubes, 0.575 mL of P_i were added and then 1 mL of thawed cells were added. The test tube was then filled to the top with media prepared previously and then capped tightly. The cultures were placed in an incubator at 30°C.[11] The incubator had a glass front to it and a lamp with an incandescent light bulb was directed into the incubator.

The cells did not grow strongly and we faulted the media used and decided to make fresh stocks for our media. It was also noted that the cells had an ending pH of 7.6 which is a bit high for growth.[3] A 1000X Trace Elements stock was created by adding the following to a 1 L plastic container: 0.190 g $CoCl_2x6H_2O$, 0.100 g $MnCl_2xH_2O$, 0.170 g $CuCl_2x2H_2O$, 0.148 g $ZnSO_4x7H_2O$, 0.025 g $NiCl_2xH_2O$, 0.025 g $VOPO_4xH_2O$, 0.002 g $Na_2WO_4xH_2O$, 0.002 g Na_2SeO_3, and 2 g Na_2EDTA. The container was filled to 1 L with distilled H_2O (ddH_2O). A biotin stock was created by adding 1 mg to 1 L of ddH_2O. A calcium stock was created by adding 1 g $CaCl_2$ to 100 mL ddH_2O. A magnesium stock was created by adding 2.5 g $MgSO_4x7H_20$ to 100 mL ddH_2O. A supplemental stock solution was created by adding 0.028 g H_3BO_3, 0.2 g Na_2EDTA, 0.04 g Fe (III) citrate, and 0.01 g Na_2MoO_4 to 100 mL ddH_2O.

The media was created by adding together 1 mL 1000X Trace Elements stock, 1 mL biotin stock, 10 mL $CaCl_2$ stock, 10 mL $MgSO_4xH_2O$ stock, and 10 mL of supplemental stock. To this was added 4 g malate, 1 g NH_4Cl, and 2 g MOPS buffer and then the mixture was diluted up to 1 L and placed in an air tight bottle to prevent contamination.

A fresh stock of P_i was created by adding 20 g KH_2PO_4 to 100 mL ddH_2O. The phosphate had a hard time dissolving until the pH was adjusted to 7 at which time the phosphate salt went into solution. This stock is a 40X phosphate stock which was used for all inoculations as noted below. It is worth noting that the phosphate is autoclaved separate from the media as it will precipitate out of solution if autoclaved with the media.

A 1 mL inoculant was taken from the cells that weren't growing and was added again to the 23 mL test tubes. The same amount of P_i was added as done previously and the tubes were filled to the top with media. Four subcultures were made—two were placed in the incubator with the incandescent light bulb while the other two were placed in a shaker hood set at 30°C exposed to lights emitting infrared light at 840 nm (see Figure 3).

After preliminary testing to see if the cells could indeed grow in these conditions, the cells were subcultured a third time into smaller test tubes that held 9.25 mL. To six of these tubes, 0.231

mL P_i were added and then the tubes and media were autoclaved. The tubes were inoculated with 1 mL inoculants from one of the cultures from the incubator (incandescent light) and then filled to the brim with media. Absorbance readings were taken immediately after inoculation using a Klett reader. Two of the cultures were placed in the incubator under incandescent light as a positive control, one was placed in a dark incubator set at 30°C with no light as a negative control, one was placed in a shaker hood set at 30°C as another negative control, and two were placed in the shaker hood with three light sources that emit infrared light at 850 nm.

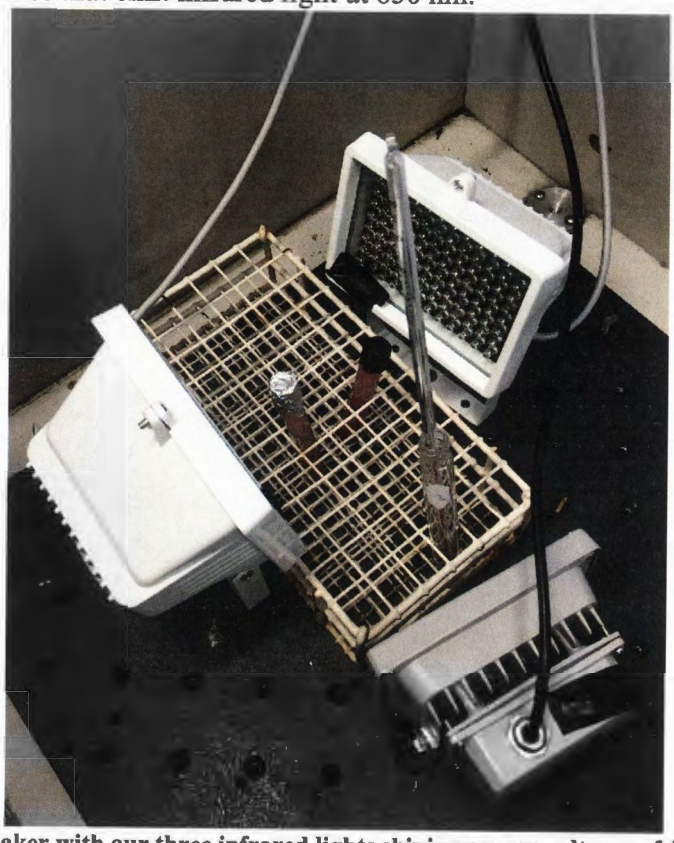


Figure 3. A picture of our shaker with our three infrared lights shining on our cultures of *R. rubrum*.

Freezer stocks were created after growth in the third inoculation by placing 1.6 mL of cells from the cells grown on incandescent light in a 2 mL cryovial with 0.4 mL of glycerol.

Methodology for enrichments are based off of our preliminary cultures on pure strains of *R. rubrum*. Media was prepared as explained above, however, two variations of media were also created to test different aspects of the bacteria. First, a media was created for growth of the bacteria on different gaseous carbon sources that would be added later to solution.[12] To accomplish this, the media was prepared without adding any malate to solution. This media was used to create cultures in serum bottles that ranged from 60-95 mL. The cultures to test for growth on different carbon sources were constructed by adding 30 mL of media that does not contain malate to the serum bottles. The inoculants for the enrichments were dirt samples that were gathered from a shallow pond. Because the dirt is not sterile, none of the equipment was sterilized before use. To the serum bottles with media, .75 mL of P_i were added. Then, 5 mL inoculants of dirt were used in each serum bottle. The experiment was performed in duplicate so eight serum bottles were prepared for four different carbon sources. The serum bottles were capped and crimp-sealed shut

so they were air tight. Using a gas manifold, the bottles were de-gassed and Ar was placed inside the bottles to make the cultures anaerobic. The carbon sources were then added using syringes through a rubber stopper in the top of each serum bottle. The carbon sources used were CO, methane, ethylene, and acetone. Each carbon source was added in excess except acetone which was added as a liquid. Only 0.05 mL of acetone was added to each culture. When the carbon sources were added, 5 mL of CO_2 were also injected into each culture.

An oxygen trap was used in each culture to ensure that the environments were strictly anaerobic. Mini test tubes were placed inside of each serum bottle before it was closed and de-gassed. A mixture of indigo carmine (IDS) and dithiothreitol (DTT) was prepared by adding 0.0134 g IDS and 0.7712 g DTT to 50 mL of ddH_2O thus making a 100 mM DTT and 0.5 mM IDS solution. DTT is a common reducing agent that is used frequently in biochemical reactions.[13] When oxidized, it forms a very stable ring structure making it highly reactive with oxygen species. IDS is a pH indicator as well as a redox indicator and is used as a food colorant.[14] It exists as either a deep blue or yellow color in solution. The yellow color indicates that IDS is in a reduced state. When mixed together in water, the two compounds appear as a blue solution. When the solution was made, it was quickly capped and kept in an anaerobic environment. DTT will slowly begin to reduce IDS until it has become completely reduced. The high level of DTT in solution means that there will still be free DTT in solution that is able to reduce any other molecules that are in solution. In our research, it would react extremely quickly with any oxygen that is in the air. As DTT reacts with oxygen, there will be less DTT in solution to reduce the IDS making the solution turn blue. The purpose of this solution was, therefore, two-fold. First, it was used as a reducing agent to react with any excess oxygen that may have been in the anaerobic cultures. Second, it was used as an indicator to make sure that our cultures were anaerobic. If they were yellow, they were anaerobic.

The second media that was created was made to test the nitrogenase activity of *R. rubrum*.[3] It is well known that this bacteria not only photosynthesizes, but also maintains the ability to fix atmospheric nitrogen into ammonia. It has been found that organisms that utilize this metabolic pathway are capable of using the amino groups from glutamate as a substrate in production of ammonia. Because of this, a media was made that did not contain NH_4Cl (which inhibits nitrogenase activity) and instead contained glutamate. Nitrogenase has a nickel metallo-center so this media was supplemented with 1 mL of a 100 mM $NiCl_2$ stock solution. This media was used to inoculate enrichments in 9.25 mL test tubes. These samples were inoculated with dirt and grown under the incandescent light.

The enrichments that showed bacterial growth were subcultured using the same protocol as outlined above for the original enrichment. From the original enrichment, 4 mL inoculants were taken to subculture.

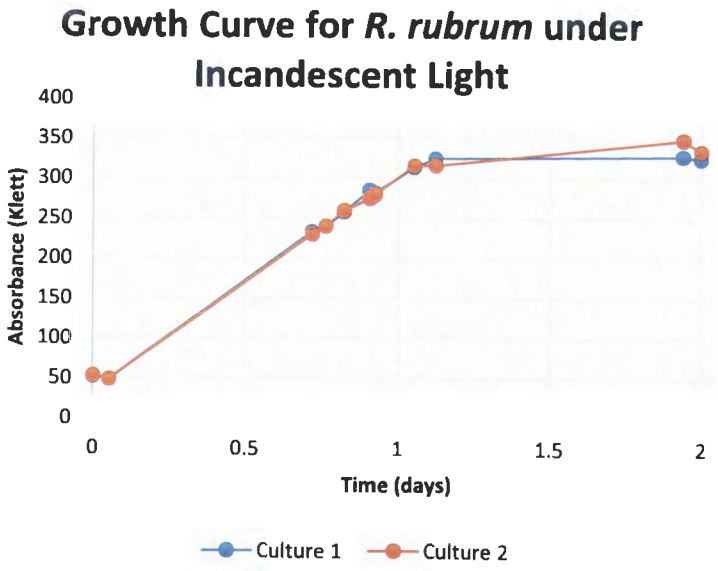
Results

This experiment was divided into five distinct subcultures. The first subculture was the cells from the freezer stock being inoculated into media stock that was determined to be old. As noted above, the cell growth was stunted and absorbance readings were not taken as the cells were visibly not growing well in this media.

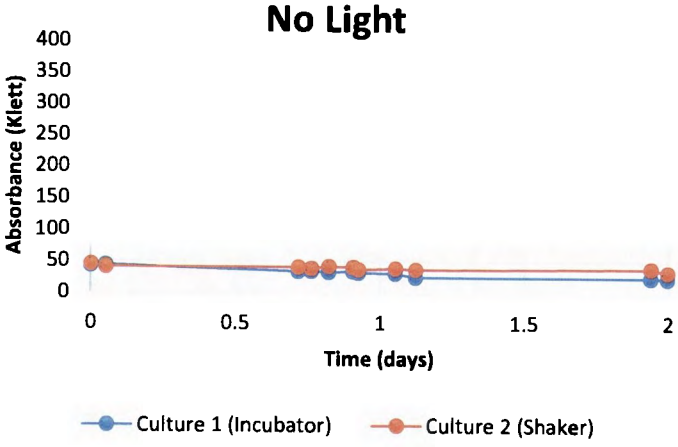
The second subculture was made by using the freshly created stocks described previously to make liquid media. The inoculation was taken from one of the cultures in the incandescent light incubator. Klett readings were taken immediately after inoculation and were generally in the range of 49-59 Klett. The cells were allowed to grow for several days and strong growths were seen. Klett readings were taken again at the end of the growths (approximately three days later) and were generally in the range of 310-338 Klett for both the infrared and the visible light cultures. The dark cultures were in the 27-31 Klett range at the end of the experiment.

The second subculture was taken as a preliminary test to make sure that our infrared lights were working correctly and that growths could actually be seen. We discovered that one of our infrared lights was dimmed for an unknown reason and the culture placed directly by this light did not grow as strong as the other infrared culture. This was fixed using an infrared sensor before continuing on to the third inoculation.

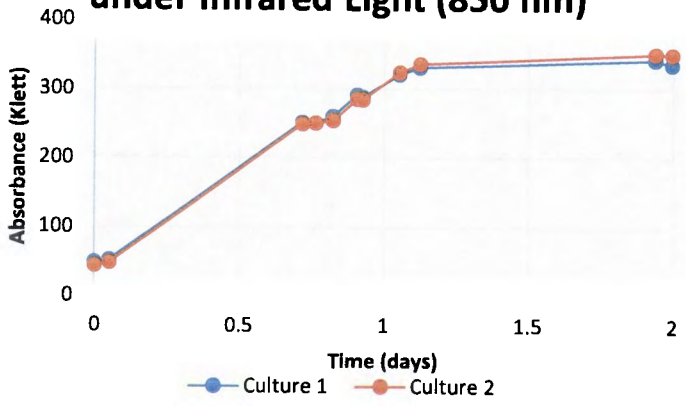
The third inoculation was taken from culture #1 in the light control incubator. As described above, 1 mL inoculants were placed in the 9.25 mL test tubes. Six test tubes were prepared and placed in the same environments as in the second subculture. Data was collected using a Klett reader and the data was graphed as seen in Figure 4. It was also noted that previous findings regarding the pigment of the bacteria were illustrated.[15] *R. rubrum* grown in culture initially grows aerobically and is clear. As time passes, all of the oxygen is used up and the bacteriochlorophyll start working causing the cells to turn a distinctive red-purple color (see left picture in Figure 5).



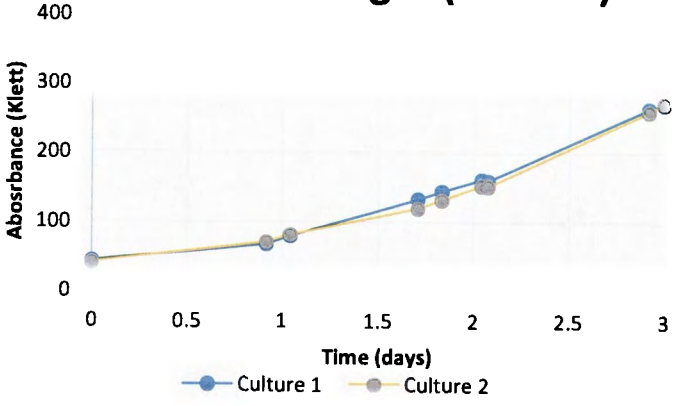
Growth Curve for *R. rubrum* with No Light



Growth Curve for *R. rubrum* under Infrared Light (850 nm)



Growth Curve for *R. rubrum* under Infrared Light (940 nm)



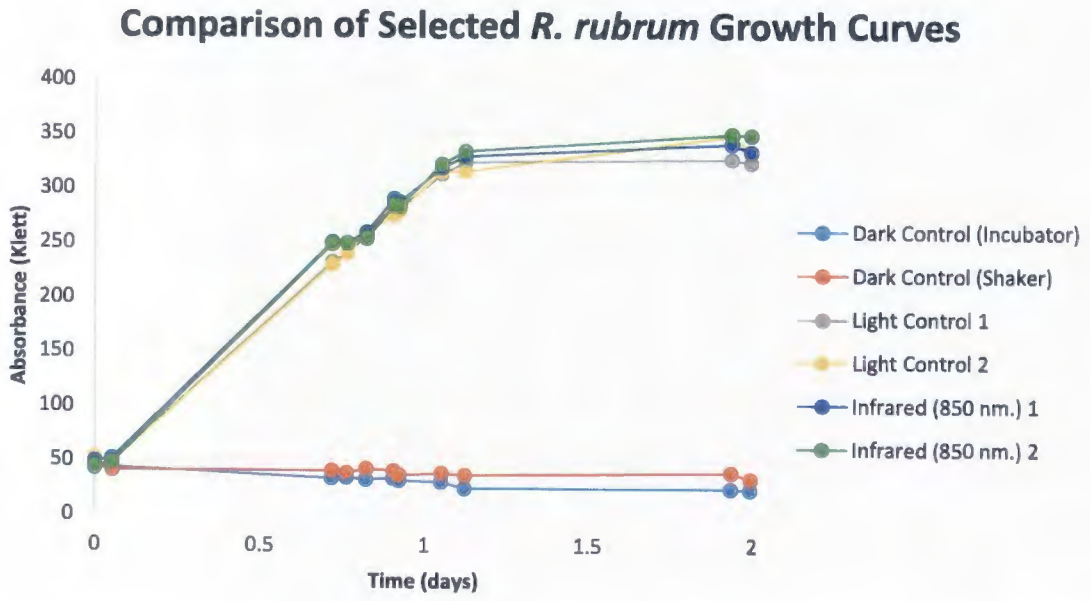


Figure 4. Bacterial growth curves for *R. rubrum*. Each experiment was completed in duplicate. The bottom-most graph is a compilation of the same data presented in the positive control, the negative control, and the infrared culture at 850 nm.

Figure 5. The left picture shows the pigment difference between a non-photosynthetic culture (left) and a photosynthetic culture (right) both grown on incandescent light. The right picture shows the pigment difference between bacteria grown at 850 nm (left) and 940 nm (right).

In addition to these results, two additional test tubes were inoculated and placed in a separate shaker hood with different infrared lights that emit light at 940 nm (see Figure 6). Only preliminary data from these lights were gathered (see Figure 4). It was found that the cells grew

more slowly at this different wavelength. In addition to this, the pigments of the grown cells were much darker red for the cells grown at 940 nm when compared to cells grown at 850 nm and in the visible spectrum as shown below (see right picture in Figure 5).

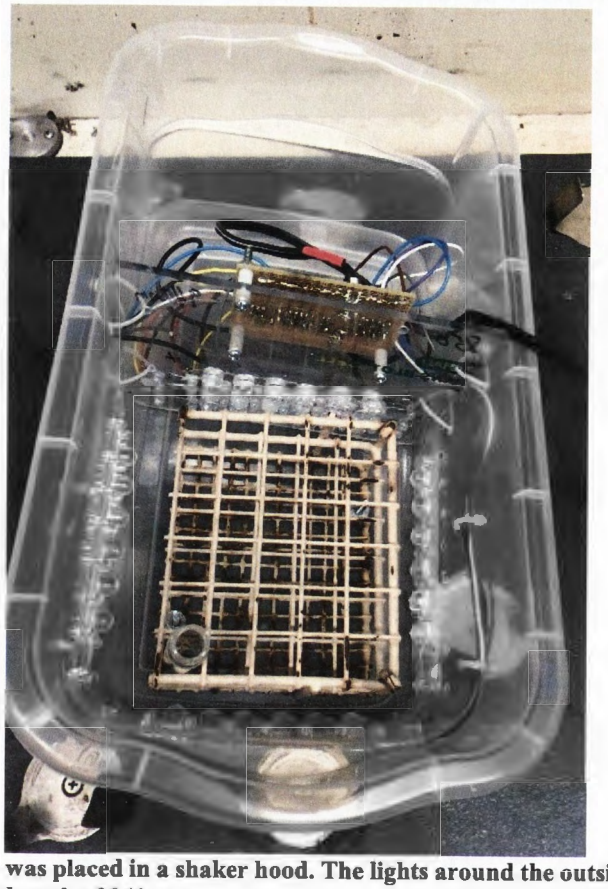


Figure 6. An apparatus that was placed in a shaker hood. The lights around the outside of the plastic container emit infrared light at a wavelength of 940 nm.

The fourth round of inoculations was the enrichments that were started with dirt inoculants. Enrichments take a long time to grow and currently, we are trying to subculture growing enrichments to try and isolate a strain of bacteria from the dirt samples gathered. Currently, we have no data from these experiments as our work is on-going. However, we are excited to have noted that it appears that bacteria are growing in the two cultures that contain acetone as the carbon source (see figure 6). The culture that was going most strongly has been subcultured and we are waiting to see if the cells will continue to grow. Our hope is to be able to subculture this growing bacteria several more times and then plate out the bacteria so that we can isolate a colony of bacteria to grow up and eventually to identify and perform a variety of other tests on.

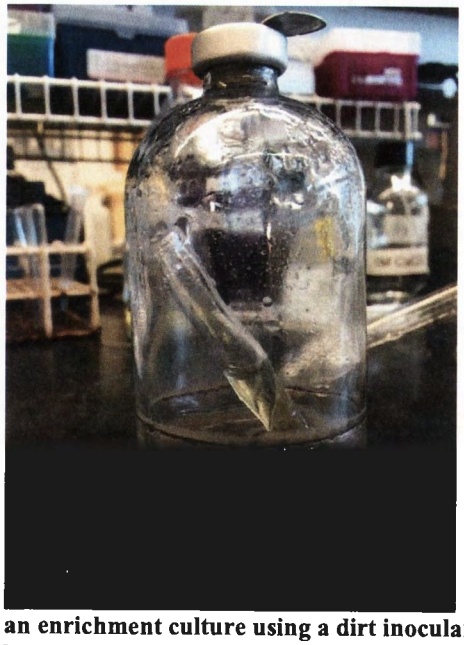


Figure 6. Serum bottle containing an enrichment culture using a dirt inoculant. The media is very turbid. This is partially due to bacterial growth and partially due to the dirt enrichment. Note the test tube in the middle that contains the yellow IDS/DTT mixture denoting that the culture is anaerobic.

The fifth round of inoculations was our glutamate/malate media which does not contain any ammonia in the media components. We have been able to perform several subcultures of the bacteria that has been growing in these cultures from dirt enrichments. These cultures were grown on visible light and are not as pertinent to our overall hypothesis. Instead, our hope is to conduct a de-repression assay on these bacteria as described above to test the activity of nitrogenase in relation to the photosynthetic activity of these cells. This is also an on-going experiment and will be conducted while we wait for the enrichments growing on infrared light to grow.

Discussion

The results support our hypothesis that *R. rubrum* can grow purely on infrared light. There could exist a body of water where algae and other plant life cover the entire surface of the pond. This plant life would strip the water below the surface of the pond of all visible light as well as all oxygen which are needed for growth for a variety of organisms. In these conditions, *R. rubrum* could thrive and grow in these darker environments by utilizing anaerobic photosynthesis off of the infrared light that is not absorbed by the algae. Our findings show that *R. rubrum* could thrive just as well on pure infrared light when compared to growth on visible light in these environments given that a carbon source, such as malate, is available.

We further hypothesize that there are other carbon sources that *R. rubrum* might have access to in these dark, anaerobic environments. Work has been done on the growth of *R. rubrum* when it utilizes carbon monoxide as the source of electrons for photosynthesis. We will monitor *R. rubrum's* growth on infrared light while utilizing carbon monoxide and other carbon sources to conduct photosynthesis.

In regards to the experiment, there were several factors taken into consideration that were not mentioned in the methods section of the report. Firstly, the experiment was designed to minimize the amount of visible light in the dark and infrared experiments to ensure that the cells were growing on pure infrared light. To ensure this, the cultures placed in the dark as a negative control were placed in two different locations. The dark culture in the incubator was an outright attempt to prove that a light source is needed for growth of *R. rubrum*. The second dark culture was placed in a shaker that was identical to the shaker where the infrared cultures were placed, but was not the same shaker. This was done to ensure that no ambient visible light was making it into the shakers. This helps to further support our findings that the cells in the infrared shaker were grown on pure infrared light without any ambient interference.

The Klett reader used is a measure of the absorbance of the cell cultures. As is seen in the results of this experiment, the Klett increases over time which represents growth of the cells. A lot of work has been done to create a direct correlation between Klett and the number of cells/mL for *E. coli* cells.[16] This conversion for *R. rubrum* will not be handled in this report as it is not the focus of the research. However, the calibrated Klett machine does have a correlation between Klett and absorbance as follows:

$$A_{600} = 0.0126 \text{ (Klett)} - 0.0377$$

This was originally found by determining that 200 Klett is equal to an Optical Density (O.D.) of 2.5. Assuming that 0 Klett = 0 O.D., the above equation can be derived.

Much work has previously been done to support the methods in this research. Gaps in understanding regarding the use of elements such as CO_2 or the purpose of carbon sources in metabolism have been handled extensively in the primary literature previously cited in this report. Suffice it to say for this report that the experiment procedures are based off of minimal growth conditions that are *REQUIRED* for bacterial growth. There is no direct way of determining whether or not specific growth conditions in and of themselves limit growth of *R. rubrum*. However, we conclude with some certainty that specific carbon sources are more usable for infrared photosynthesis while other carbon sources are not.

Conclusions and Future Work

The findings surrounding the fact that *R. rubrum* can grow on pure infrared light help us to better understand the underlying metabolic processes that this bacteria employs to survive. By using these new techniques, it is now possible to conduct experiments to further understand processes such as the use of CO in the dark as a metabolite.[12]

The purpose of our research has been to develop a novel methodology that can be used to hopefully isolate new strains of bacteria using pure infrared lights. By design, this research is mostly focused on methodology specifics and the "why" behind what has been done in the lab. The implications of this research will be pursued heavily with its application to future research experiments. Our ability to measure the success of this research will come as we are able to isolate new strains of bacteria from our enrichments. At that point, the possibilities and implications become endless. Research will be conducted to identify and classify any new strains of bacteria. Proteomes can be obtained to learn more about the metabolic strategies of these new bacteria. Research can be conducted to better understand how bacteria are able to switch between different metabolic strategies and these can help us to understand how this bacteria picked the specific niche of being aquatic anaerobes to thrive in. Studies can also be conducted in other fields such as physics where we can study and begin to understand the sources of infrared light that photosynthetic bacteria utilize to survive.

These studies along with countless others, can all lead to a better understanding of microbial metabolism. As we master these concepts, we can begin to find beneficial uses of this bacteria to aid humanity such as food for animals or fertilizers. Metabolism mastery can unlock these scientific discoveries.

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Author's Biography

Jordan Lee Wilkes is a Cache Valley native and the last child in his family to earn his undergraduate degree. He will earn a Bachelors of Science degree in Biochemistry with a minor in Biology. While at Utah State University, Jordan has served in many capacities not only within the lab but within various other organizations. Jordan served as a member of the USU A-Team and as a Peer Mentor. He also was the Vice President of the USU Science Council and taught as an Undergraduate Teaching Fellow. Jordan will be graduating this spring Cum Laude distinction as well as distinction as an Undergraduate Research Scholar.

While attending school Jordan married the love of his life, Kelsie Wilkes. Jordan and Kelsie currently live in Logan, UT and are working full time jobs while they wait to hear back from medical schools that Jordan has applied to. Jordan plans to go on and earn a professional degree and become a pediatrician.